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DEVELOPMENT OF A "PRIMITIVE" SEA URCHIN (*EUCIDARIS TRIBULOIDES*): IRREGULARITIES IN THE HYALINE LAYER, MICROMERES, AND PRIMARY MESENCHYME[1]

THOMAS E. SCHROEDER

Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington, U. S. A. 98250; and Discovery Bay Marine Laboratory, University of West Indies, Discovery Bay, Jamaica

Abstract

The normal embryonic development of the cidaroid sea urchin *Eucidaris tribuloides* from fertilization to the late two-armed larval stage is described with the aid of light and electron microscopy. Unusual developmental features include a virtual absence of a hyaline layer, irregularities in the number and size of micromeres, and a total lack of early mesenchyme (the so-called primary mesenchyme). These and other features are unlike developmental characters in the euechinoids ("modern" sea urchins). They are discussed with reference to the phylogeny of sea urchins. *Eucidaris tribuloides* may exhibit "primitive" developmental patterns representative of the cidaroid ancestor which was common to all living sea urchins.

Introduction

Nearly all extant sea urchins are members of the Subclass Euechinoidea, whose fossil record extends about 175 million years into the past. Although their precise phylogenetic origin is somewhat uncertain (Nichols, 1962; Durham, 1966), members of this subclass probably diversified rapidly soon after the Permo-Triassic extinction about 225 million years ago (Fig. 1). The ancestor of the euechinoids was a cidaroid sea urchin (Order Cidaroida), whose relatively undiversified survivors compose the Subclass Perischoechinoidea. The ancestral genus may well have been *Miocidaris*, the only echinoid definitely known to have survived the extinction. Today cidaroids form a relatively inconspicuous part of the marine fauna, usually occupying deep-water tropical habitats.

Since living cidaroids closely resemble the cidaroid ancestors of modern euechinoids, some of their characteristics, including any distinguishing features of their embryonic development, may be regarded as "primitive." Hence, study of cidaroid development may have phylogenetic relevance. In the well-studied euechinoids, development typically proceeds through a regular and characteristic 16-cell stage marked by a tier of 8 mesomeres in the animal hemisphere, a tier of 4 large macromeres, and a vegetal tier of 4 small micromeres (Hörstadius, 1973; Okazaki, 1975). Later, two populations of mesenchymal cells form. Micromeres produce primary mesenchyme, which emigrates from the wall of the late blastula into the blastocoel to give rise to the larvel skeleton. Secondary mesenchyme arises from the vegetal-most derivatives of the macromeres (veg_2); it emerges near the tip of the archenteron when the archenteron is about halfway invaginated, and forms the coelomic and other mesoderm.

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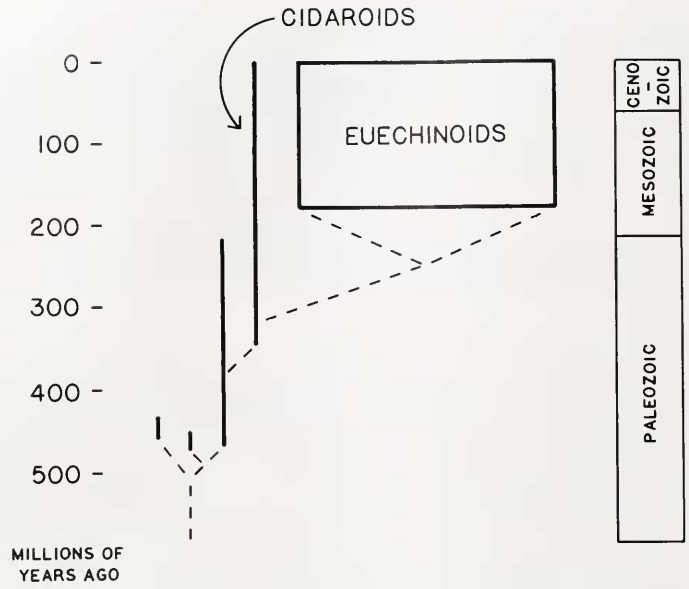

FIGURE 1. The phylogenetic relationship of cidaroid sea urchins to recent sea urchins (euechinoids), as simplified after Duncan (1966). The only known survivor of a massive extinction at the end of the Paleozoic was a genus of cidaroids; it may have been the common ancestor for both euechinoids and present-day cidaroids, or the ancestral cidaroid for euechinoids may have been earlier. Since the extinction, euechinoids have diversified extensively while cidaroids have not. Cidaroids which have survived to the present remain relatively "primitive."

Reproduction and development in cidaroids have received rather little attention (Holland, 1967; McPherson, 1968; Pearse, 1969). Tennent (1914, 1922) reported that embryos of *Eucidaris tribuloides* do not produce any primary mesenchyme, yet the larvae do form skeletons. The present article re-examines the development of this Caribbean cidaroid, using specimens from a shallow sub-tidal reef very near Tennent's original collection site. The study discusses certain unusual features of this urchin's development in light of the evolution of the more regular developmental pattern exhibited by other sea urchins.

MATERIALS AND METHODS

Eucidaris tribuloides adults (Fig. 2) were collected in Discovery Bay, Jamaica, during the second week of March 1980. They were typically found beneath or beside slabs of broken coral at depths of 2–3 m. Shedding of gametes was induced in the laboratory by intracoelomic injection of 0.5 *M* KCl. Of 80 adults collected, gametes were obtained from 4 males, 11 females, and 1 hermaphrodite. The remaining individuals contained no ripe gametes. Eggs washed twice in seawater were fertilized with dilute suspensions of sperm. Cultures were maintained in dishes in a sea-table or on a bench-top (26°C) or in an air-conditioned room (22°C). Seawater in the culture dishes was changed daily.

Eggs and embryos were photographed using Nomarski differential interference contrast microscopy, or bright-field microscopy between crossed polarizers for recording birefringence in the larval skeletons. To avoid compressing specimens during observations or photography, cover slips were supported on small daubs of

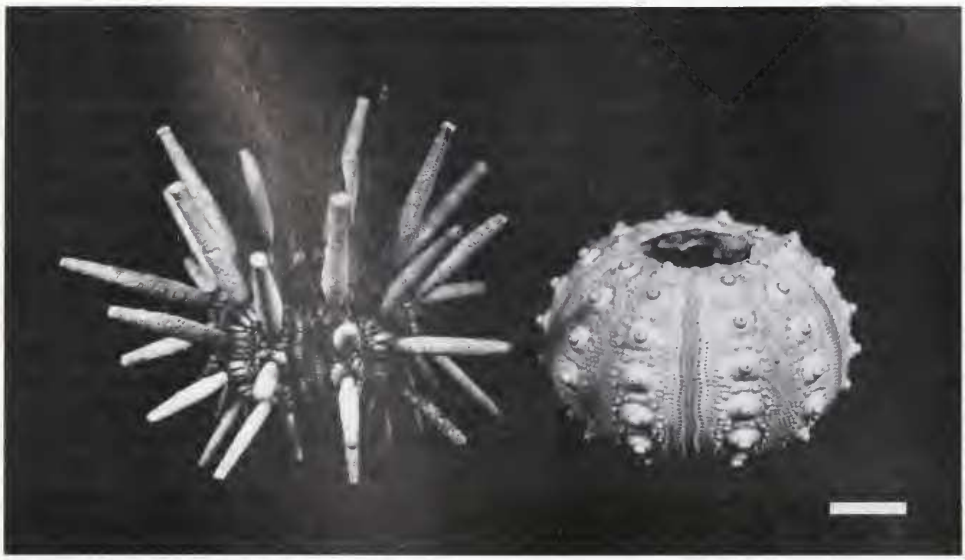

FIGURE 2. Life-size photograph of a small dried *Eucidaris tribuloides* (left) and the cleaned test of a large specimen (right). Note the heterologous spines which characterize this group. Scale bar = 1 cm.

modeling clay. Swimming specimens were immobilized with a small quantity of glutaraldehyde just before they were photographed.

Specimens were fixed in 2.5% glutaraldehyde–0.09 *M* sodium cacodylate–69% seawater pH 7.2 for 1 h, rinsed briefly in 0.2 *M* sodium cacodylate–50% seawater, and post-fixed in 1% osmium tetroxide–0.09 *M* sodium cacodylate–69% seawater for 1 h. They were then dehydrated and embedded in Epon for transmission electron microscopy, or critical point dried from CO_2 and coated with gold-palladium for scanning electron microscopy.

On occasion, fertilization envelopes were removed by pre-treating unfertilized eggs in 1 m*M* aminotriazole (Showman and Foerder, 1979) and then repeatedly passing them through a narrow-bore pipette about 30 min after fertilization. The eggs were then restored to seawater. This procedure successfully removed about 10% of the fertilization envelopes and caused the remainder to collapse. Development was normal after this treatment, even in the continuous presence of aminotriazole for 24 h.

RESULTS

The eggs of *Eucidaris tribuloides* are about 95 μm in diameter and are quite transparent. When the eggs were fertilized, a narrow perivitelline space 5–8 μm wide formed beneath the elevated fertilization envelope. A hyaline layer could scarcely be discerned at any time. Figure 3 shows stages of development at 26°C. First cleavage began at 50 min and subsequent cleavages occurred about 30 min apart. The 16-cell stage was visible at 2.5 h.

The embryos hatched at about 8 h, before the blastula could swim effectively. That natural dissolution of the fertilization envelope normally precedes active swimming was confirmed by rearing a culture directly on the microscope stage, thereby

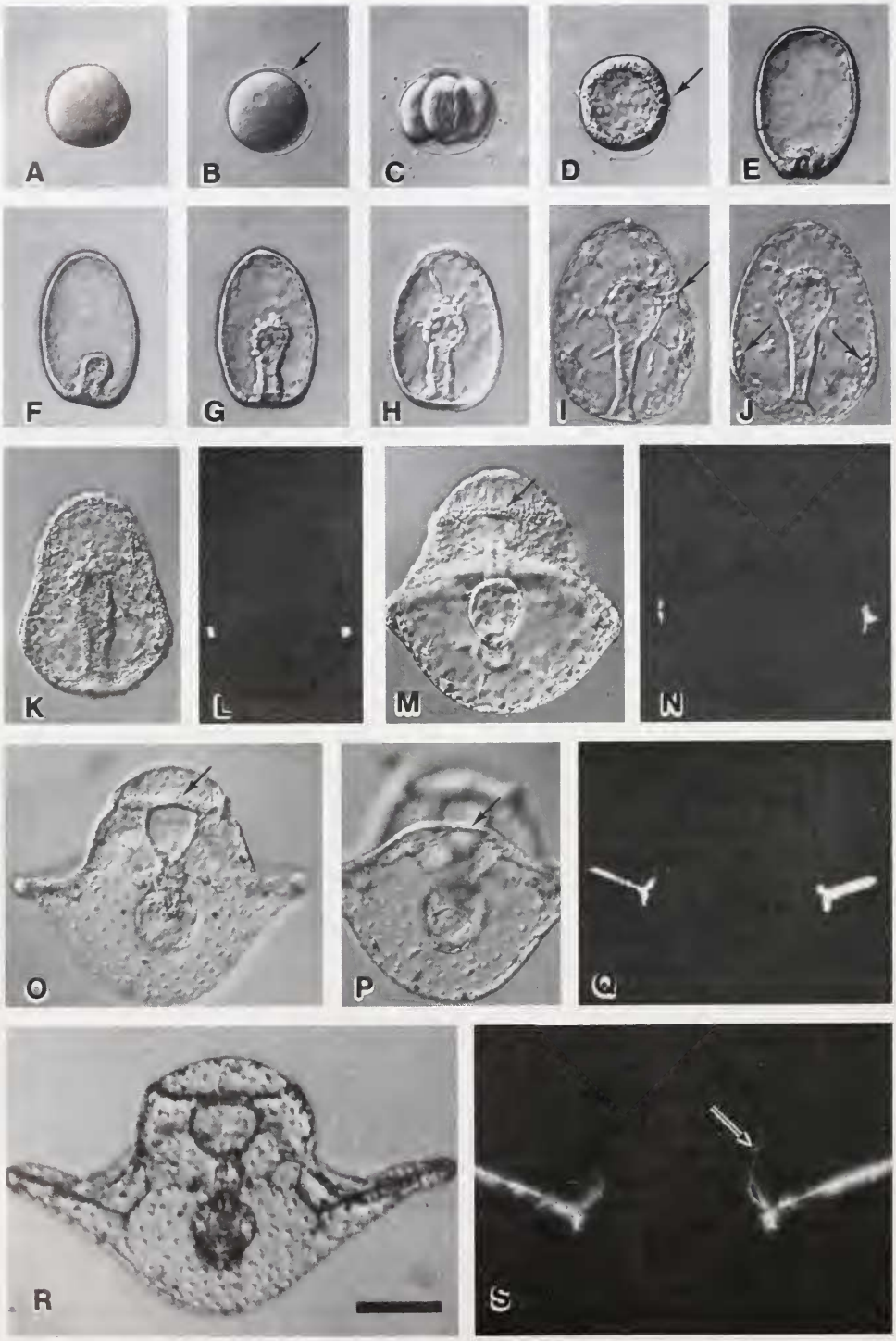

FIGURE 3. Stages of embryonic development of *Eucidaris tribuloides* cultured at 26°C. A: the unfertilized egg. B: the elevated fertilization envelope (arrow) 8 min after fertilization. C: the four-cell

ruling out the possibility that pipetting the embryos had artifically stripped off the envelopes. An apical tuft of cilia never formed after hatching. By 12 h swimming behavior was evident, but it remained sluggish throughout development.

As shown in Figure 3, the first sign of an invaginating archenteron was at about 14 h. The archenteron invaginated halfway before any sign of mesenchyme appeared. Even then, at 20 h, mesenchyme precursors were still attached to the archenteron tip. The blastocoel contained migratory mesenchyme cells by 22 h. By 40 h the archenteron contacted the stomodeum, and most of the mesenchymal cells were arrayed in the vicinity of this structure, except for two small clusters of cells situated bilaterally at the widest part of the embryo near the blastopore. By 44 h a birefringent spicule was detectable in each of these clusters. These spicules subsequently developed into the larval skeleton. In the next few days of development, the longest skeletal element was the post-oral rod, which in this species is fenestrated. Even after 7 days of culture, the slow-swimming larva was still two-armed and transverse rods did not join the bilateral skeletal elements. I never observed feeding, even when larvae were offered a suspension of mixed algae. A rudimentary ciliated band that appeared at about 70 h was complete by 75 h.

When eggs were cultured at 22°C, development was slowed so that the archenteron invagination began at 21 h and mesenchyme first appeared at 40 h. Despite this slow rate, the pattern of development was the same as at 26°C.

Figures 4–6 show selected aspects of development in greater detail. Figure 4 shows the virtual absence of the hyaline layer surrounding early embryos. Although microvilli about 1 μm long appeared over the entire surface a few minutes before first cleavage began, they were not obviously embedded in a detectable hyaline layer, even under completely normal conditions (Fig. 4A). When the fertilization envelope was mechanically removed, the shape of the early two-cell stage at the end of first cleavage (Fig. 4E) was considerably different from the shape when the constraining fertilization envelope was still intact (Fig. 4B). Without the fertilization envelope, the blastomeres were nearly spherical, further indicating the lack of a coherent hyaline layer. Subsequent development without a fertilization envelope resulted in disarrayed blastomeres (Fig. 4F, 5). Microvilli were readily visible by scanning electyron microscopy (Fig. 5B), even though no effort was made to remove the hyaline layer. This further attested to the virtual absence of a persistent hyaline layer.

Thin sections of eggs before and after fertilization (not illustrated) contained cortical granules that disappeared at fertilization. The contents of these cortical granules may be involved in forming the perivitelline space and in hardening the fertilization envelope, but either they contain little hyaline precursor or it readily solubilizes upon its release.

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stage at 1 h 48 min. D: the non-swimming blastula hatching out of the ruptured fertilization envelope (arrow) at 8 h. E: early stage of invagination at 14 h. F: the early gastrula at 18 h; note the absence of primary mesenchyme. G: the mid-gastrula at 20 h. H: the first signs of mesenchyme cells in the blastocoel at 21 h. I: lateral view from the right side when the archenteron tip contacts the stomodeum (arrow) at 39 h. J: the same embryo in frontal optical section showing two clusters of mesenchymal cells (arrows), which give rise to the primary spicules of the larval skeleton. K and L: the primary spicules appear as birefringent spots (L) in the early prism larva at 44 h. M and N: the advanced prism with developing ciliated band (arrow) near the mouth and enlarged tri-radiate spicules (N) at 69 h. O, P and Q: different focal levels of the young two-armed pluteus larva with a single complete ciliated band (arrows) and elongating post-oral rods (Q) at 75 h. R and S: the two-armed larva remains virtually unchanged between 100 and 180 h of culture, conceivably for lack of food; the antero-lateral rods of the skeleton (arrow) have formed in this 180 h specimen. A–S at the same magnification. Scale bar = 100 μm.

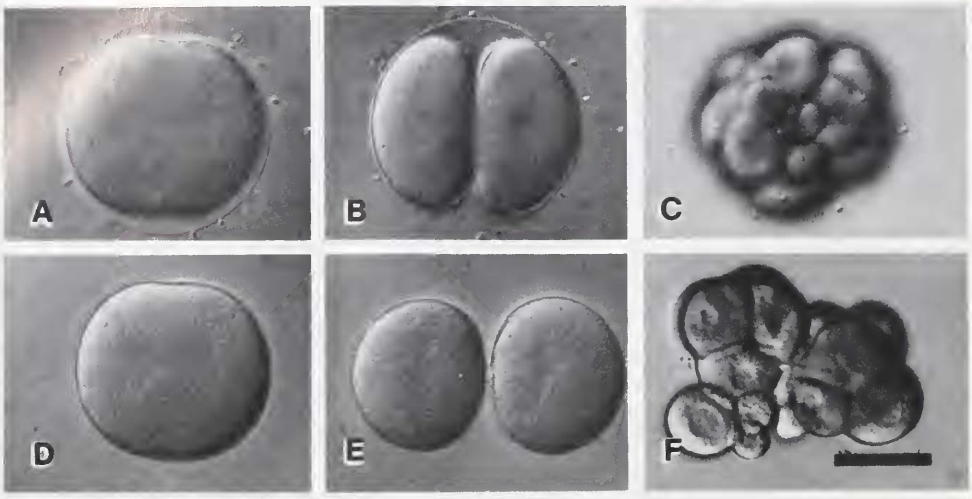

FIGURE 4. Details of early development when the fertilization envelope is intact (A–C) and after its removal (D–F). By light microscopy there is no evidence of a hyaline layer, even at the onset of first cleavage (A and D) when microvilli can be detected. When it is present, the fertilization envelope constrains the first two blastomeres into a roughly spherical shape (B), but without it the blastomeres separate widely (E). At the 16-cell stage there is an irregular number of micromeres (C and F) and the blastomeres are disarrayed; this is especially evident after the fertilization envelope has been removed and the embryo assumes a roughly rectangular configuration (F). A–F at the same magnification. Scale bar = 50 μm.

Figures 4 and 5 show the pattern of blastomeres at the 16-cell stage. Although micromeres formed, there were rarely 4 of them. Usually there were two or three micromeres of variable size and an equivalent number of macromeres, as judged by cell size. The remaining cells were all about the same size ("mesomeres"). Micromeres developed similarly, with or without fertilization envelopes, except that in the latter cases the embryos assumed a somewhat rectangular configuration (Fig. 4F, 5A), apparently because they were less confined. In scanning electron micrographs the surface of micromeres typically had few microvilli (Fig. 5B).

Closer inspection of blastulae and gastrulae (Fig. 6) confirmed that there was no evidence of mesenchyme cells in the blastocoel up to the mid-gastrula stage. When the archenteron was about one-third invaginated, some thin cytoplasmic projections reached into the blastocoel from cells near the tip (Fig. 6A). One-micron sections at a slightly later stage showed that the blastocoel was still devoid of cells, but filled with a loose meshwork of fibrils. Cells began to migrate into the blastocoel began at this stage (Fig. 6B).

DISCUSSION

This study confirms previous reports of the slow development of *Eucidaris tribuloides* (Tennent, 1914 and 1922; McPherson, 1968) and the unusual development of mesenchyme (Tennent, 1914 and 1922). Development in this species differs from that of more familiar euechinoid sea urchins in the following ways: (1) development is extremely slow; (2) very little hyaline material forms as the result of cortical granule release at fertilization; (3) blastomeres are separated

widely after the first few cleavages, and are in disarray thereafter; (4) micromeres at the 16-cell stage are irregular in size and number, with corresponding effects on the appearance of macromeres; (5) hatching occurs before the blastula is very mobile; (6) there is no apical tuft of cilia; (7) no mesenchyme enters the blastocoel until the archenteron is halfway invaginated; (9) swimming at all stages is extremely slow; (10) the two halves of the larval skeleton remain unjoined at the midline for long periods.

Available evidence on the development of other cidaroids indicates that the pattern in *Eucidaris tribuloides* is representative of development in the Order Cidaroida generally. Prouho (1887) and Mortensen (1938) reported extremely slow development in other cidaroids. According to Mortensen (1938), the early blastomeres of *Prionocidaris baculosa* are widely separated, suggesting that the hyaline is reduced in this species, as it is in *Eucidaris tribuloides*. He also mentions that "in the 16–32 cells stage the cells were arranged in a rather unusual way, forming a two-layered plate" (Mortensen, 1938, p. 13). This recalls the rectangular appearance of embryos in Figures 4 and 5. He also reports that the number and distribution of the large macromeres and the small micromeres is variable, and that often no size difference can be detected (Mortensen, 1937).

The timing of mesenchyme migration into the blastocoel in cidaroids deserves additional clarification and comparative study. The absence of early mesenchyme in *Eucidaris tribuloides* may resemble the situation in *Prionocidaris baculosa*, according to a drawing of an early gastrula by Mortensen (1938). On the other hand, Prouho (1887) illustrates a similar stage of a Mediterranean cidaroid in which primary mesenchyme is specifically identified; the embryo also has an apical tuft, unlike *Eucidaris tribuloides*. The organism in the study was called *Dorocidaris papillata*, but has been renamed *Cidaris cidaris* (Mortensen, 1928).

The peculiar development of the mesenchyme in *Eucidaris tribuloides* suggests several possible interpretations. According to one view, it may indicate that this species entirely lacks any micromere-derived primary mesenchyme and that all migratory cells in the blastocoel are really secondary mesenchyme. This is not necessarily inconsistent with formation of a larval skeleton, since it has been shown (Hörstadius, 1973, p. 48, 50, and 61) that larvel spicules can still form in euechinoid embryos from which the micromeres have been experimentally removed. Apparently, cells originating from the veg_2 can organize spicules under certain circumstances.

This interpretation may be extended by postulating that *Eucidaris tribuloides* does not segregate the determinants of the micromeres and larval skeleton into the vegetal regions of the early embryo. Accordingly, the irregularities of the micromeres, the lack of primary mesenchyme, and the absence of an apical tuft could all be manifestations of an animal-vegetal axis that was imperfectly established at early stages (Schroeder, 1980).

According to an alternate interpretation, two separate populations of mesenchyme cells could happen to migrate simultaneously and subsequently sort out in the blastocoel. This would tend to obscure the distinction between primary and secondary mesenchyme, which is usually based on the times of migration rather than on cell lineage.

To determine which interpretation is correct may require experimental manipulations similar to those performed on euechinoids (Hörstadius, 1973). Such an approach would determine the role of micromere-derived cells in the pattern of

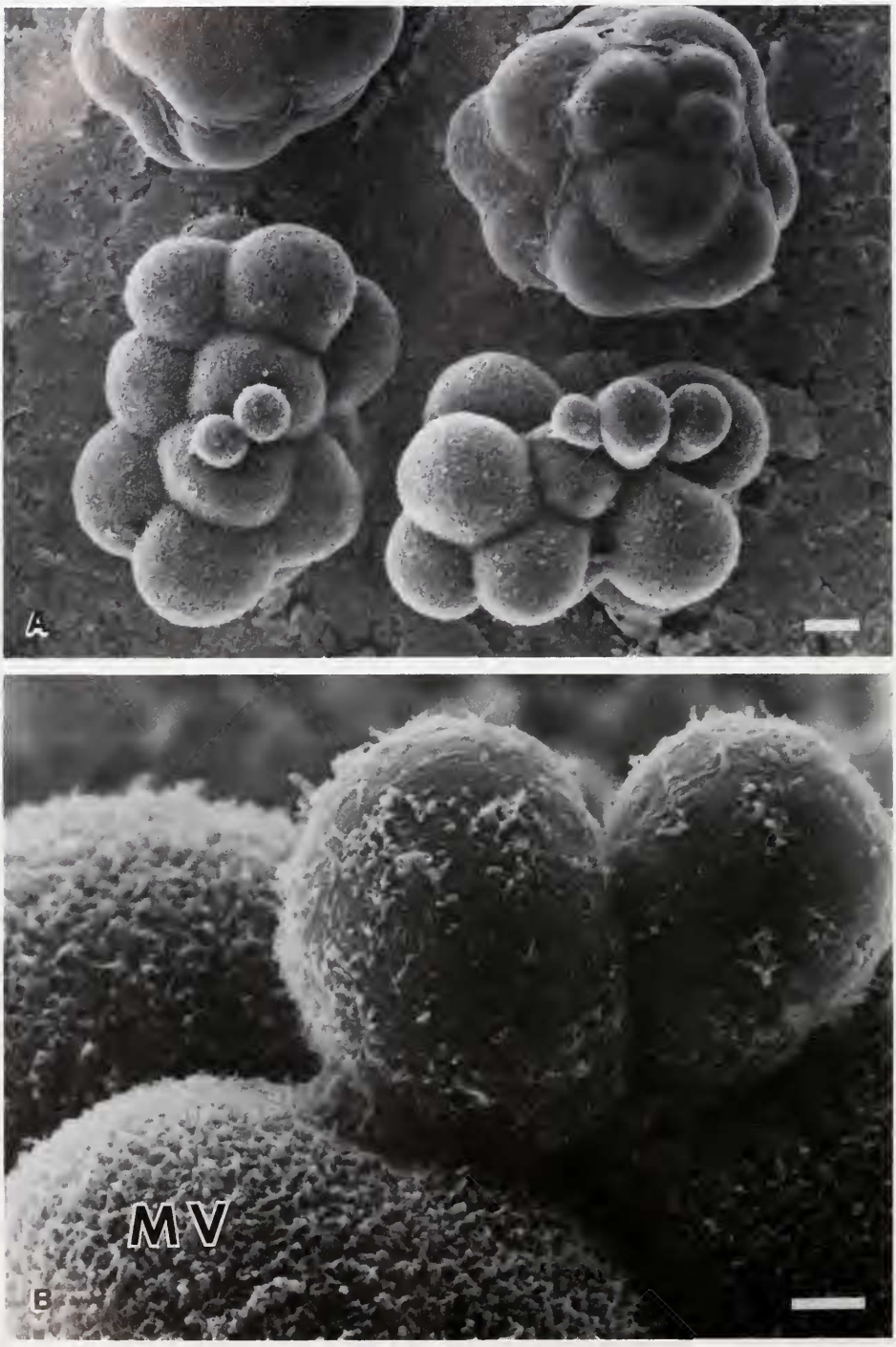

FIGURE 5. Scanning electron micrographs of embryos at the 16-cell stage, with fertilization envelopes removed (except for the two at the top of the figure). Embryos at this stage typically have 2 or

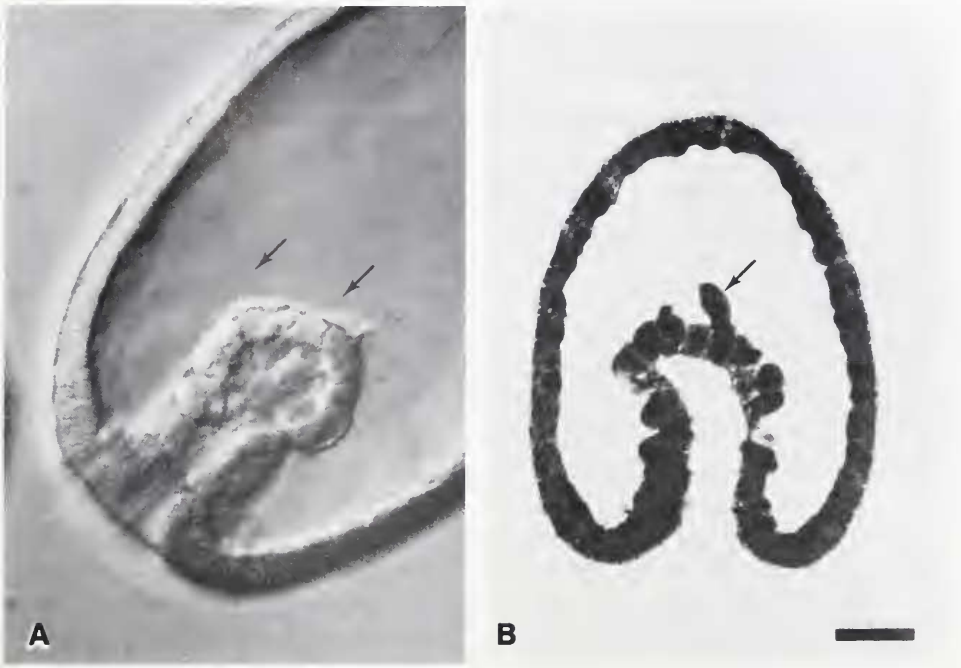

FIGURE 6. In *Eucidaris tribuloides*, mesenchyme cells do not enter the blastocoel until the middle stage of archenteron invagination. A Nomarski micrograph (A) of an early gastrula at 18 h reveals the lack of mesenchyme cells in the blastocoel; however, a few thin cytoplasmic processes (arrows) extend from the tip of the archenteron where mesenchyme cells will soon emerge. A 1 μm Epon section (B) of a mid-gastrula at 19 h (slightly shrunken during preparation) shows a precursor cell of the mesenchyme (arrow) shortly before it dissociates from the archenteron. A and B at the same magnification. Scale bar = 20 μm.

development. If skeleton-formation in cidaroids depends upon macromere-veg_2 cells rather than micromeres, this fact could help elucidate the phylogeny of the distinctive developmental pattern of euechinoids: It would indicate that the micromere-primary mesenchyme-larval skeleton system is a relatively recent evolutionary development.

Certain key developmental events differ greatly among the classes of the Phylum Echinodermata (Table I). But how these patterns relate to phylogeny is not clear. Only the euechinoids of Class Echinoidea have four micromeres at the 16-cell stage; these give rise to primary mesenchyme and then the larval skeleton. Ophiuroids do not form micromeres, but they still proliferate an early mesenchyme before archenteron invagination (Olsen, 1942). They also form a very prominent early larval skeleton very similar to the echinoid skeleton. Holothuroids (Oshima, 1921; Maruyama, 1980), crinoids (Seeliger, 1893), and asteroids (Dan-Sohkawa *et al.*, 1980) do not form micromeres or early mesenchyme, but some skeletal elements do appear in holothuroid embryos and crinoid larvae. Asteroid larvae never develop skeletons.

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3 micromeres, which vary in size, and an overall rectangular configuration (A). Microvilli are clearly visible on the larger blastomeres (B, MV), along with a minute amount of hyaline layer material. Scale bars = 10 μ (A) and 2 μm (B). Shrinkage has occurred during preparation.

TABLE I

Distribution of developmental features among the echinoderms. "Early mesenchyme" forms before or at the time of archenteron invagination. "Late mesenchyme" appears in the second half of archenteron invagination.

Taxon of Echinoderm	Developmental features			
	Micromeres at 16-cell stage	Early mesenchyme	Late mesenchyme	Larval skeleton
Class Holothuroidea	0	−	+	+
Class Echinoidea				
Subclass Euechinoidea	4	+	+	+
Subclass Perischoechinoidea (*Eucidaris tribuloides*)	1-4	−	+	+
Class Crinoidea	0	−	+	+
Class Asteroidea	0	−	+	−
Class Ophiuroidea	0	+	+	+

Several of the questions that arise in comparing the developmental pattern of *Eucidaris tribuloides* with development in other echinoderms (Table I) focus on the validity of homologies between mesenchyme populations and the cellular localization of skeleton-forming potential. A deeper analysis of this issue is necessary to understand the place of *Eucidaris tribuloides* in the Class Echinoidea, or the phylogenetic relationship between classes that might be discerned from embryonic development.

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